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A time-series of methane and carbon dioxide production from dairy cows during a period of dietary transition

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Abstract

Emissions from dairy farms are contributing to the increased concentrations of greenhouse gases which are linked to recent climate change. Altering diets has been proposed as a greenhouse gas mitigation strategy in dairy systems. The magnitude of mitigation and the time taken for cows to adapt to new diets has not been comprehensively quantified. Methane (CH_4) and carbon dioxide (CO_2) produced by dairy cows was measured for six weeks using the sulphur hexafluoride tracer technique following a change in diet; from barley straw and protein supplements to grazed grass. CH_4 and CO_2 production increased linearly as the animals adapted to their new diets, however, production did not reach an asymptote six weeks into the grazing period. This suggested that metabolic activity and greenhouse gas emissions may not have been at their maximum. There was substantial variation between individuals with high emitting cows producing four times more CH_4 than low producing cows. Cows which produced greater amounts of CH_4 consistently also produced greater CO_2. We demonstrate that feeding regime plays an important role in determining greenhouse gas emissions and we highlight that transition periods in greenhouse gas models and future experiments must be sufficiently large to allow for adaptation.

Keywords: climate change, dairy, dry period, enteric methane, greenhouse gases, transition.

1. Introduction

Atmospheric concentrations of carbon dioxide (CO_2) and methane (CH_4) have increased substantially over the past 150 years. Although CO_2 is the most influential driver of climate change (~50% of total radiative forcing, RF), net CO_2 emissions from agriculture are small by comparison to those of CH_4 (IPCC 2013). CH_4 is the second most influential greenhouse gas (~28% of RF) with between 21 and 25 times the global warming potential (GWP) per gram of CO_2 (IPCC 2013). Livestock farming produces approximately 7.1 gigatonnes of CO_2 equivalents annually (GT CO_2eq) – 15 % of anthropogenic greenhouse gas emissions (FAO, 2013). Enteric fermentation by livestock produces 2.8 GT CO_2eq of CH_4 each year, with 77% being produced by cattle (FAO, 2013).

Dairy farming produces approximately 2 million tonnes of CO_2eq worldwide each year (this value includes milk production, processing and transportation, and meat production from dairy-related culled animals) - 4% of total anthropogenic greenhouse gas emissions (FAO 2010). There is substantial variation between emissions from different regions, production systems and cow breeds. CH_4 produced by individual cows have been shown to range from 137 g d^{-1} to 431 g d^{-1} (Lassey et al 2007) with approximately 96% of CH_4 production being the result of the fermentation of carbohydrates by microbes in the rumen and intestine (Mcginn et al., 2006). CO_2 is also produced within the rumen by microbial respiration as well as by respiration by the cows themselves with one study recording CO_2 production per cow ranging from 9,900 g d^{-1} to 14,680 g d^{-1} (Kinsman et al 1995). Rates of CH_4 and to a lesser extent CO_2 production are under the control of the activity rate, population size and community composition of enteric microbes (Lettat et al 2013). Factors which can modify enteric microbial activity include the composition of feed and quantity of feed intake, the breed or genotype of the animal and environmental conditions such as location or temperature (McAllister et al 1996). However, the direction of the response in CH_4

production to changes in temperature have been shown to be both positive and negative (McAllister et al 1996), and is presumably context dependent.

Enteric CH_4 production can be modified by cow diet directly due to a change in microbial substrate availability or indirectly via a change in rumen pH (Bath et al 2013). O'Neil et al (2011) compared groups of cows fed either a mixed ration (containing maize silage, grass silage, concentrate, barley straw and molasses) or a diet consisting solely of grass, recording increased mean CH_4 production per cow from the mixed ration fed group compared with the grass-fed group – likely due to increased feed intake and microbial substrate availability. Reducing the digestibility of feed also increases CH_4 production (e.g. by increasing fibre content) since the residence time of feed within the rumen is increased and the opportunity for methanogenesis by the microbial population is elevated (Brask et al 2013). Conversely, increasing the digestibility of feed (e.g. by increasing starch or glucose content) reduces CH_4 production since feed moves through the digestive system more rapidly and the opportunity for methanogenesis by the microbial population is reduced (Janssen 2010).

Changing cattle diets can influence the environmental footprint, productivity and profitability of livestock production systems (Lee and Roberts, 2015). The identity of the crops grown to feed livestock as well as farm management practices, such as soil tillage, can influence carbon fluxes and associated greenhouse gas emissions (Al-Kaisi and Yin, 2004). Weather conditions, soil erosion and leaching also modifies the carbon budgets of livestock farms (Comino et al 2017) and can lead to a re-distribution of carbon stocks (Nie et al 2016).

There are few studies which have measured changes to CH_4 produced by cows over time following a change in diet. One such study demonstrated that mean CH_4 increased between weeks four (314 g d^{-1}) and ten (333 g d^{-1}) following a change in diet (O'Neill et al 2011). However, we are not aware of any study which has investigated how the production of CH_4

varies over time whilst cows adapt to grazing conditions and none which have also measured CO_2. We sought to contribute to this knowledge gap by regularly measuring CH_4 and CO_2 produced by 12 non-lactating dairy cows following a change in diet; from barley straw and protein supplements fed indoors to outdoor grazing of grass. The following hypotheses were tested: (1) CH_4 and (2) CO_2 production would increase over time as cows adapted to grazing; (3) Cows would produce more CH_4 and CO_2 per kg of liveweight over time and (4) CH_4 and CO_2 production would asymptote within six weeks of the change in diet.

2. Materials and methods

2.1. Site and weather conditions

The study was carried out at Scotland's Rural College (SRUC) Dairy Research Centre, Dumfries, South-West Scotland (3^o 35 W, 53^o 03 N) during May and June. Air temperatures ranged from 4.6 ˚C to 19.8 ˚C during the seven week study period, with a mean of 6.2 ± 0.7 hours of sunshine per day. Weekly mean soil temperatures (5 cm depth) increased from 12.2 ˚C at the start of the study to 16.3 ˚C at the end. Rainfall varied from 0.1 mm d^{-1} in the driest week to 25.6 mm d^{-1} in the wettest (Table 1). Weather data were obtained from an on-site weather station.

➔ Table 1

2.2. Animals and experimental design

The study group consisted of 12 non-lactating Holstein-Friesian dairy cattle (mean age 5.5 ± 2.8 years, mean liveweight 576 kg ± 51 kg). Two of the animals were freemartin heifers, with the remaining ten cows maintained in the follicular phase of the reproductive cycle for the duration of the study, to minimise any changes to the animals during the experiment. This was achieved by means of Progesterone Releasing Intra-vaginal Devices (PRIDS: Ceva Animal Health Limited, UK) administered prior to commencement of the study. Cows were housed indoors over the winter and fed a diet of barley straw in preparation for taking part in the study. In the four weeks prior to commencement of the study cows were fed a diet of unrestricted barley straw and each cow also received 3 kg d^{-1} of 18% protein concentrate. The feeding of protein supplements prior to the grazing treatment was in line with best practice for straw-fed high yielding dairy cattle.

Cows were separated into two sub-groups. This allowed a one week delay in the start date between the two sub-groups. This staggered start was incorporated in the study design as a means of reducing the impact of single-day climate effects and variation in forage quality. Cows were allocated to one of the two groups by separating the animals into matched pairs based on age and weight. Individuals were then allocated into one of the two sub-groups at random. This ensured that each sub-group was balanced for age and weight at the start of the experiment (Group 1 - mean age ± standard error; 5 ± 3 years; mean liveweight; 566 ± 53 kg; Group 2 - mean age; 6 ± 3 years, mean liveweight; 586 ± 52 kg).

On day one of the measurement phase of the study sub-group one were turned out to pasture and allowed to graze freely for 23 hours per day without supplementary feeding for a six week period. Cows were brought inside for one hour a day. This allowed the renewal of SF_6 tracer equipment and for the cows to be weighed. One week later sub-group two was also allowed to graze the pasture under the same management regime for a period of six weeks. Measurements of CO_2 and CH_4 produced by each cow and measurement of cow weight were carried out daily for the first ten days at pasture, then three days per week from weeks three to the conclusion of the study. As a result daily greenhouse gas production and liveweights for each cow was measured 22 times.

2.3. Pasture composition, productivity and nutritional quality

The grazing area was a 4 ha pasture dominated by a perennial ryegrass (*Lolium perenne*) sward (approximate cover > 95%). The pasture was sub-divided into six smaller paddocks by means of a movable electric fence. Cows were moved between fields every two days to allow for the grass to re-grow before cows returned to graze again twelve days later. This regime aimed to retain a consistent grass height across the study period and ensured that grass availability was unrestricted and did not influence feed intakes. Sward height was measured daily using a sward stick, placed randomly at 50 locations across the pasture (mean sward height throughout the study = 10.0 ± 0.9 cm).

Each day five grass samples (~25 g) were collected from random locations across the field and harvested to ground level. Samples were bulked on a weekly basis and analysed for nutritional quality. Nutritional quality measurements were dry matter (DM), gross energy (GE), metabolisable energy (ME), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and hemicellulose content (HC). DM content was assessed by weighing 5g of plant material, drying this material for 48 hours at 60 °C and comparing dry and fresh weights. CP was measured by Kjeldahl digestion using sulphuric acid and analysed by steam distillation using a Gerhardt-Vadopest system (Gerhardt Vadopest 6, Germany). NDF, ADF and HC were measured using modified neutral and acid detergent analysis following the methodology of Van Soest et al. (1991). GE and ME was measured by conventional wet chemistry, as outlined by AOAC (2002).

2.4. Methane and carbon dioxide emissions measurements

CH_4 and CO_2 production was measured using the sulphur hexafluoride (SF_6) tracer technique (Johnson et al., 1994). A permeation tube bolus (brass 15 mm OD, 45 mm long, 55 g) with a semi-permeable Teflon membrane (5 mm diameter) and halter containing the inert tracer gas SF_6 was introduced to the rumen of the study animals. Prior to deployment, the individual release rates of SF_6 from 24 boluses were measured by weighing at daily intervals over a period of five weeks, during which time the tubes were held at 39 ^{o}C in an anaerobic nitrogen environment to simulate rumen conditions (Berndt et al 2014). Changes to bolus weight was plotted against time with the 12 boluses which exhibited the strongest linear relationship (highest r^2 value) being selected for use in the experiment (mean loss rate = 1.44 ± 0.04 mg SF_6 d^{-1}). Boluses were administered to the animals three weeks prior to the measurement period to allow for acclimation and to minimise the probability of non-linear release of SF_6 during the measurement period. After the experiment, all of the boluses were recovered post mortem and inspected for blockages or any other damage. There was no evidence of any blockages and no evidence of any non-linearity in SF_6 release rates in the six weeks prior to the start of the experiment or during the experiment. It was therefore assumed that, once ingested by the animals, each permeation tube remained in the rumen releasing SF_6 gas at a constant rate according to its individual release signature.

CH_4 production rates (F_{CH4}) were estimated using equation 1 and CO_2 production rates (F_{CO2}) were estimated using equation 2 where F_{SF6} is the known release rate of SF_6 from the permeation tube (g s^{-1}) and where C_{SF6}, C_{CH4} and C_{CO2} are the concentrations (g m^{-3}) of the three gases in the exhaled air.

$$F_{CH4} = F_{SF6}\ C_{CH4}/C_{SF6} \quad (1)$$

$F_{CO2} = F_{SF6}\ C_{CO2}/C_{SF6}$ (2)

Exhaled air from the animal was sampled from the area around the nostrils using flexible tubing held in place by a halter and connected via a metal capillary tube to a closed v-shaped PVC canister secured behind the cows head. The canisters were evacuated using a vacuum pump prior to use and the shut off valves were opened on attachment to the cows to commence air sampling. This arrangement allowed exhaled air to be sampled continuously for 24 hours until the valves were closed. On removal of the canisters from the animals new evacuated canisters were attached to sample the next 24 hour period. The contents of the removed canisters were diluted with nitrogen (mean dilution: 3.59 ± 0.05), decanted into sub sampling tubes constructed from metal and glass, then transported to the laboratory for subsequent analysis using an HP5890 Series II gas chromatograph (detection limits: $SF_6 <$ 0.005 ml l^{-1}, $CO_2 <$ 0.199 ml l^{-1} and $CH_4 <$ 0.00126 ml l^{-1}) using an electron capture detector for SF_6 and a flame ionisation detector for CH_4 and CO_2. Dilution factors were recorded for each sample and measured CO_2, CH_4 and SF_6 concentrations adjusted accordingly.

2.5. Statistical analysis

Relationships between daily CH_4 and CO_2 production (g d^{-1}) and experimental duration as well as relationships between CH_4 and CO_2 production per gram of cow liveweight (g d^{-1} kg^{-1}, CH_4 / LWt and CO_2 / LWt) and experimental duration were identified for the group using maximum-likelihood linear mixed effects models (LME, Pinheiro and Bates 2000). The relationship between CH_4 and CO_2 production was also tested using LME. In all models, each cow was treated as a random effect with duration treated as a fixed effect. This random effect structure allowed us to account for our time series, where several measurements of CH_4 and CO_2 emissions were taken from an individual animal over the course of the study. The optimal shapes of the relationships were identified by means of transforming our response data using logarithmic and quadratic transformations, comparing LME model outputs with those generated by untransformed data using AIC (Akaike's Information Criterion). AIC represents an alternative for calculating measurements of the explained deviance to the more conventional r^2 values which cannot be calculated with LME models. In all cases the linear relationship had the lowest AIC value and was selected (Crawley 2007). The equations of fitted lines from these analyses represent both the mean rate of increase in a stated parameter over time (gradient) and the mean absolute value of the stated parameter on day one following the change in diet (intercept).

Relationships between CH_4 and CO_2 production and experimental duration, and CH_4 and CO_2 production per gram of liveweight and experimental duration were tested for each individual cow using linear regression (LR). Relationships between CH_4 and CO_2 production and cow weights were also tested using LR for each day since the change in ration. LR was used in these instances since these data were not nested – identifying relationships between CH_4 and CO_2 production and duration for each cow and between CH_4 and CO_2 production and liveweight on each day, respectively. Relationships between grass sward quality (DM, GE, ME, CP, NDF, ADF, HC) and CH_4 and CO_2 were also tested using LR using mean weekly values for sward quality and gas production. Due to the staggered design of the experiment, separate analyses were computed for CH_4 and CO_2 production for sub-groups one and two against their respective grass sward quality measurements. The optimal shapes of the relationships were identified by means of transforming response data using logarithmic and quadratic transformations and comparing LR model outputs with those generated by untransformed data using r^2. In all cases the linear relationship had the highest r^2 value and was selected (Crawley, 2007). All analyses were computed using R v3.0.1 (R Core Team, 2013).

3. Results

3.1. Group greenhouse gas emissions

Total group production of both CO_2 ($t = 4.0$, $P < 0.001$) and CH_4 ($t = 7.4$, $P < 0.001$) increased linearly over the experimental period and following the change in diet (Figure 1). Mean production of CO_2 per cow increased from 11,429 g d^{-1} on day one to 16,825 g d^{-1} on day 38 (LME: $CO_2 = 142d + 11,429$, $P < 0.001$). This represented a mean increase in CO_2 production of 142 g d^{-1} or a rise of 47% over the 38 day experimental period.

Mean production of CH_4 per individual cow was lower than CO_2 throughout the study, increasing from 272 g d^{-1} on day one to 386 g d^{-1} on day 38 (LME: $CH_4 = 3d + 272$, $P < 0.001$). Mean production of CH_4 per cow also increased at a slower rate than CO_2; increasing by 3 g d^{-1} or 42% over the 38 day experimental period.

➔ Figure 1

There was a positive linear relationship between CO_2 production and CH_4 production over the experiment ($t = 32.5$, $P < 0.001$, Figure 2). Cows which produced large amounts of CO_2 also produced large amounts of CH_4 and days which produced large amounts of CO_2 also large amounts of CH_4, with a 1g increase in CH_4 associated with a 44 g increase in CO_2 (LME: $CO_2 = 44 * CH_4$, $P < 0.001$).

➔ Figure 2

3.2. Forage nutritive quality

Forage nutritive quality metrics generally increased by the end of the study, with DM (+19%), GE (+3%), ME (+12%), CP (+20%) and ADF (+18%) all increasing between days 1 and 38 (Table 2). However, NDF (-3%) and HC (-24%) declined over the same period. None of these metrics increased or decreased consistently over the study period. Across all of the metrics for forage quality the number of weeks in which the metric increased compared with the previous week and the number of weeks in which the metric declined was approximately equal (range = 2 – 4 weeks increasing and range = 2 – 4 weeks decreasing).

➔ Table 2

Weekly mean CH_4 production was not related to any of the forage quality metrics for the first sub-group of cows, which commenced the experiment in week one (t = -0.1 – 1.5, P = 0.2 – 0.9). However, weekly mean CH_4 produced by sub-group two, which commenced the experiment in week two, were negatively correlated with weekly mean NDF content (t = -2.8, $P < 0.05$, $r^2 = 0.6$). All other forage quality metrics were not related to CH_4 over the experimental period for this sub-group (t = -1.75 – 1.71, P = 0.15 – 0.73). In addition, none of the forage quality metrics were related to mean weekly CO_2 production over the experimental period for sub-groups one (t = -0.7 – 1.4, P = 0.1 – 0.7) or two (t = -1.5 – 1.4, P = 0.2 – 0.9).

3.3. Cow liveweights

Mean cow weight within the group increased from 576 ± 13 kg (mean ± standard error) on the first day to 583 ± 17 kg on day 38, representing a 1% increase. These increases were idiosyncratic and on a weekly basis mean group weight declined by 0.5% between weeks one and two, increased by 1% between weeks two and three, decreased by 2.4% between weeks three and four and then increased by 2.5% and 3.2% between weeks four and five, and between weeks five and six, respectively.

There were no relationships between cow weight and CH_4 or cow weight and CO_2 production on any of the first 23 and 17 days of the study, respectively (Table 3). On day 31, CH_4 increased linearly with cow weight, with each 1 kg increase in cow weight representing a 1.6 g d^{-1} increase in CH_4 emissions ($t = 3.8$, $P < 0.001$). CO_2 also increased linearly with cow weight but only on days 22, 23 and 31. On these three days, each 1 kg increase in cow weight represented a 62 g d^{-1} ($t = 2.5$, $P < 0.05$), 70 g d^{-1} ($t = 2.3$, $P < 0.05$) and 62 g d^{-1} ($t = 3.0$, $P < 0.05$) increase in CO_2 production, respectively.

➔ Table 3

Over the study period, the mean amount of CH_4 ($t = 6.6$, $P < 0.001$, Figure 3) and CO_2 ($t = 3.6$, $P < 0.001$) produced per kg of cow liveweight increased linearly. In the case of CH_4, the group produce a mean of 0.5 g d^{-1} kg^{-1} on day one rising by 0.005 g kg^{-1} each day. After 38 days, the group was therefore producing mean CH_4 of 0.7 g d^{-1} kg^{-1}. In terms of CO_2, the group produced a mean of 20.9 g d^{-1} kg^{-1} rising more steeply on a daily basis, by 0.25 g d^{-1} kg^{-1}. The group was therefore producing mean CO_2 of 30.1 g d^{-1} kg^{-1} by day 38.

➔ Figure 3

3.4. Individual cow greenhouse gas emissions

Eight of the twelve cows showed a linear relationship between experimental duration and CH_4 production ($t = 2.3 – 6.2$, $P = <0.001 – 0.04$, Table 4). Two of these eight cows were the freemartin heifers. Variation between cows which produced low CH_4 and those which produced high CH_4 was substantial, by approximately four fold in terms of CH_4 on day one and by approximately four fold in terms of the rates of increase in CH_4 over the experimental period. For example, production of CH_4 on day one ranged from 116 g d^{-1} for cow eight to 510g d^{-1} for cow nine, with the rates of increase in CH_4 over the 38 day experimental period ranging from 1.3g d^{-1} for cow eight to 5.3g d^{-1} for cow seven.

Rates of CH_4 production per kg of liveweight also increased linearly for the same eight cows ($t = 2.2 – 6.1$, all $P = <0.001 – 0.04$, Table 4) alongside absolute CH_4 increases. However, the rates of increase in CH_4 production per kg liveweight increased more slowly over time and with a reduced range compared with absolute CH_4 production – ranging from 0.003 and 0.008 or by a factor of approximately 2.7.

CO_2 production was also linearly related to experimental duration for the same eight cows ($t = 2.6 – 8.9$, all $P <0.001$). The ranges of emissions on day one were greater for CO_2 than for CH_4, ranging from 4,611g for cow two to 20,971g for cow six or by a factor of approximately five. Rates of increases in CO_2 production over the experimental period were also moderately greater for CO_2 than CH_4, ranging from an increase of 89g d^{-1} for cow eight to an increase of 367 g d^{-1} for cow one. This represented an approximately four-fold difference.

➔ Table 4

The rank order from highest to lowest producing cow was relatively consistent over the 38 days with the standard deviation of the rank order for individual cows, representing each cows mean distance from their mean rank, ranging from 0.6 to 3.0 and from 1.3 to 2.4 for daily CH_4 and CO_2 emissions, respectively (Table 5).

➔ Table 5

4. Discussion

Production of CH_4 and CO_2 from both groups of cows increased over time following the shift in their diets; from straw to grazed grass. This increase was likely to have been driven by changes in feed chemical composition and increased feed intakes by the animals, as has been reported in studies elsewhere (e.g. McAllister et al 1996; O'Neill et al 2011). This finding is supported by comparison of the nutritive quality of barley and grass, with the DM content of grass around 4 times lower than that of barley straw indicating that a greater volume of grass would have been required by the cows to satisfy their nutritional demands. Since the cows were retained in the follicular phase and were not pregnant or lactating, the results obtained were unlikely to have resulted from the lifecycle of the animals during the experiment.

Elevated CH_4 and CO_2 production over the experimental period may have been partially driven by weight gains of the animals thus increasing their capacity for forage intake and metabolic activity. However, on the majority of sampling occasions there was no relationship between cow liveweights and the quantity of CH_4 or CO_2 that was produced. Those occasions where significant relationships were obtained may have been statistical artefacts, since the error associated with weighing the animals was large. Although cow liveweights increased between week one and week six, these gains were idiosyncratic. Despite these small liveweight gains (~1%), CH_4 and CO_2 production increased rapidly and the cows became more efficient producers of CH_4 and CO_2 per kg of liveweight. This suggests that weight gains were not key determinants of changes to the magnitude of CH_4 and CO_2 production and also highlights that cow weights were not good predictors of total CH_4 and CO_2 production.

Whilst nutritional differences between the two contrasting diets are likely to have been important, shifts in grass quality following the transition to grazing are unlikely to have played a major role in driving the linear increases in CH_4 and CO_2 production. An exception was a negative relationship between NDF concentrations and CH_4; however, this relationship was relatively weak and only significant for the second sub-group of cows. Typically NDF is positively related to CH_4 production (Lee et al 2017) and therefore this relationship is also likely to be a statistical artefact. Grass quality varied throughout the study but none of the grass quality metrics increased regularly (both increasing and decreasing on a weekly basis) alongside a more consistent and linear increase in CH_4 and CO_2. Non-linear release of SF_6 has been demonstrated to influence CH_4 measurements in studies elsewhere, particularly over longer periods (Lassay et al 2001). We tested all boluses for linear release rates over the five weeks prior to the experiment. The magnitude of change in CH_4 when compared with the relatively small error generated by non-linear release over the six week measurement period and careful inspection of boluses post-mortem means that it is unlikely that non-linearity of SF_6 release has driven the relationships presented in this study.

Mean CH_4 production increased per cow from 272 g d^{-1} during week one to 386 g d^{-1} during week six, producing quantities of CH_4 which were consistently greater than those produced by grass fed cows in Ireland (251 g d^{-1}, O'Neill et al 2011), Canada (270 g d^{-1}, McCaughey et al 1999) and New Zealand (159 g d^{-1} – 202 g d^{-1}, McCaughey et al 1997). By the sixth week of the study the group was producing CH_4 emissions which were only moderately less than cows fed a diet of mixed ration in Ireland (397 g d^{-1}, O'Neill et al 2011) and greater than all but one group of grass and clover fed cows in New Zealand (137 - 431 g d^{-1}, Lassey 2007). It is likely that increased feed intake and subsequent changes to the availability or chemical composition of microbial substrate played an important role in driving elevated CH_4 production (Kebreab et al 2006). However, it has also been demonstrated that non-lactating cows lose a greater proportion of their feed intake as CH_4 than lactating cows (Bell et al 2010) and this may have contributed additionally to the high values we recorded.

Production of CO_2 was 42 - 44 times greater than CH_4 throughout the study and CO_2 also increased more rapidly than CH_4. Our estimate of average CO_2 production over the six week period (14,364 g d^{-1}) was comparable to values that were recorded using an infra-red gas analyser to measure grass-fed lactating Holstein-Friesian cows in Canada (12,055 g d^{-1}; Kinsman et al 1995) and greater than a previous study using the SF_6 tracer technique in France (8,750 – 10,496 g d^{-1}; Pinares-Patino et al 2007) providing additional support for the use of the SF_6 tracer technique to measure CO_2 production. The direction and magnitude of changes to CO_2 production provide useful insights into metabolic changes during the experiment. The rise in CO_2 production over the course of the study may be explained by increased respiration by the cows, digesting larger quantities of feed coupled with respiration by enteric microbes during rumen adaptation (McAllister et al 1996). Previous studies have shown that the SF_6 tracer technique overestimates CO_2 production, with the magnitude of overestimation recently estimated as 20 - 65 % (Pinares-Patino et al 2007). Despite this, considering the 21-25 times higher GWP of CH_4 when compared with CO_2 (IPCC, 2013), the GWP of CO_2 produced by the cows throughout the study was approximately double (200 – 210%) the GWP of CH_4 according to our measurements – greater than the maximum proposed overestimation of 65%. Although it should be noted that CO_2 emissions from agriculture are considered to be balanced by subsequent plant carbon uptake in greenhouse gas inventories (IPCC 2013), an increased efficiency of milk production per unit of CO_2 and CH_4 would reduce the overall carbon footprint of dairy farming systems.

Selective breeding studies have demonstrated that CH_4 production can be reduced by 19% - 23% if selection is based on milk production (Chagunda et al 2009) and retaining older cows can also reduce CH_4 by 3%, since more productive older cows convert feed to milk more efficiently (Bell et al 2010). Within our groups of cows there was substantial variation between individuals, with the lower producing cows producing four and five times less CH_4

and CO_2 than the high producing cows, respectively. The rank order of the highest to lowest individuals was consistent over the study and cows which produced high CH_4 also produced high CO_2. Variation was not explained by cow liveweights, cow age or grass nutritional quality and is likely to be linked to enteric conditions; where the rumen is more or less favourable for methanogenic microbial population growth and activity (McAllister et al 1996). These data quantify the potential for reductions in greenhouse gas emissions if cow selection is based on minimising CH_4 production.

CH_4 and CO_2 production continued to increase linearly throughout the six week grazing period and did not asymptote. This indicates that the increase in feed intake by the cows and/or the increase in enteric microbial activity may not have reached saturation point. Care needs to be taken in designing future livestock studies so that they are of sufficient duration to capture the full change in greenhouse gas production as animals adapt to novel feeding systems. In the absence of measured data, CH_4 production is currently estimated using predictive equations based on DM intakes, nutrient intakes and the digestibility of the diet (Mills et al 2003). It has been shown that these equations can give accurate predictions of enteric CH_4 production (Ulyatt et al 2002a; Ulyatt et al 2002b). However, our data suggests that these equations should also take into account changes to the chemical composition of feed and consider the magnitude and duration of change in greenhouse gas production.

5. Conclusions

Two groups of non-lactating dairy cows were associated with increased CH_4 and CO_2 production following a change in their diet; from straw and protein supplements to grazed grass. Both CH_4 and CO_2 production increased more rapidly and consistently than cow weight gains and forage nutritive quality indicating that production of both gases may have increased as cows adapted to the new feeding system. CH_4 and CO_2 production did not reach an asymptote over the six week grazing period, which was not expected, indicating that CH_4 and CO_2 production rates may not have reached maximum values. Predictive equations and future experiments should therefore consider the magnitude and duration of adaptation during periods of dietary transition. There was substantial variation in greenhouse gas production between individuals with our analyses highlighting that cows which produced higher CH_4 also produced higher CO_2. These data highlights that feeding regime is an important driver of greenhouse gas production, quantifies the potential for reductions in greenhouse gas production using selective breeding and also indicates that measurements of CO_2 production may serve as a useful proxy for CH_4 production by dairy cows.

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Figure 1. Linear relationships between experimental duration and carbon dioxide emissions ($t = 4.0$, $P < 0.001$; filled triangles) and methane emissions ($t = 7.4$, $P < 0.001$; filled circles) following the change in diet. Fitted lines represent carbon dioxide ($CO_2 = 142d + 11{,}429$, dashed line) and methane ($CH_4 = 3d + 272$, continuous line) as defined by LME. Values are means of 12 cows ± SE ($n = 228$ measurements)

Figure 2. Linear relationship between carbon dioxide and methane emissions throughout the study period ($t = 32.48$, $P < 0.001$). The fitted line represents this relationship, as described by LME ($CO_2 = 44 * CH_4$). Each value is a daily measurement taken from 1 of 12 cows ($n = 228$ measurements)

Figure 3. Linear relationships between experimental duration and methane produced per kg of liveweight (CH_4 / LWt , $t = 6.6$, $P < 0.001$; filled circles) and carbon dioxide produced per kg of liveweight (CO_2 / LWt , $t = 3.6$, $P < 0.001$; filled triangles) following the change in diet. Fitted lines represent methane ($CH_4 / LWt = 0.005d + 0.5$, continuous line) and carbon dioxide ($CO_2 / LWt = 0.25d + 20.6$, dashed line) as defined by LME. Values are means of 12 cows ± SE ($n = 228$ measurements)

Table 1. Weather conditions over the study period. Minimum daily air temperature (min air temp), maximum daily air temperature (max air temp), hours of sunshine and daily rainfall. Data were obtained from an on-site weather station.

Week	Min air temp (˚C)	Max air temp (˚C)	Sunshine (h d^{-1})	Rainfall (mm d^{-1})
1	4.6	13.9	6.5	25.6
2	9.1	16.9	3.8	15.8
3	6.1	17.4	9.1	7.1
4	7.4	16.8	6.8	8.9
5	10.5	18.7	6.6	5.3
6	11.8	19.8	6.2	0.1
7	10.1	17.4	4.3	2.3
Mean	8.5	17.3	6.2	9.3
SEM	1.0	0.7	0.7	3.3

Table 2. Weekly measurements and overall mean values for canopy height and herbage quality over the study period (n = 5 measurements). Metrics are dry matter (DM), gross energy (GE), metabolisable energy (ME), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and hemicellulose content (HC). Weeks + indicates the number of weeks where that parameter increased compared with the previous week and week – indicates the number of times the parameter decreased compared with the previous week. Indicative values for barley straw (Barley) obtained from Moss et al. (1990).

Week	Height (cm)	DM (g Kg^{-1})	GE (Mj kg DM^{-1})	ME (Mj kg DM^{-1})	CP (g kg DM^{-1})	NDF (g kg DM^{-1})	ADF (g kg DM^{-1})	HC (g kg DM^{-1})
1	10.8	178	18.6	11.3	207	452	227	225
2	10.9	144	18.8	11.1	207	504	245	259
3	9.6	215	18.3	10.9	194	484	258	226
4	8.4	248	18.3	10.7	235	480	227	253
5	9.8	188	19.2	11.9	269	483	223	260
6	9.8	179	19.1	12.6	256	464	222	242
7	10.8	211	19.2	12.7	250	437	267	170
Mean	10.0	195	18.8	11.6	231	472	238	234
se	0.3	12.6	0.2	0.3	11	9	7	12
Weeks +	4	3	3	3	2	2	3	3
Weeks -	2	3	3	3	4	4	3	3
Barley	-	841	18.5	-	44	799	523	276

Table 3. Linear regression analyses of relationships between cow weight (kg) and methane (CH_4) and carbon dioxide (CO_2) emissions for each day of the study (n = 12 cows). Mean liveweight for each time period are also presented (LW)

		CH_4 (g d^{-1})			CO_2 (g d^{-1})		
Day	LW (kg)	Gradient	t	P	Gradient	t	P
1	576	0.49	0.64	0.54	-2.30	-0.09	0.93
2	564	0.70	1.31	0.22	23.91	1.00	0.34
3	556	1.05	2.23	0.05	35.27	1.84	0.10
4	558	1.01	1.73	0.12	25.97	0.70	0.50
5	556	0.89	1.06	0.32	32.14	0.86	0.41
6	572	0.12	0.12	0.91	-22.88	-0.44	0.67
7	562	0.92	0.69	0.51	36.13	0.76	0.47
8	571	0.76	0.90	0.40	9.03	0.26	0.80
9	556	1.02	1.39	0.20	32.57	0.91	0.39
10	558	0.91	1.45	0.18	27.79	1.71	0.12
15	563	0.15	0.18	0.86	134.80	0.52	0.62
16	573	1.49	1.77	0.12	97.76	1.96	0.09
17	563	0.58	0.70	0.50	40.55	1.23	0.25
22	554	1.36	2.15	0.06	62.40	2.48	0.03
23	557	1.53	1.77	0.11	69.76	2.33	0.04
31	570	1.64	3.77	<0.001	62.13	2.99	0.02
38	583	1.11	2.22	0.06	49.26	1.81	0.11

Table 4. Regression analyses identifying linear relationships between experimental duration and methane and carbon dioxide emissions for each cow (n = 22 measurements). Methane emissions per day (CH_4), methane emissions per kg of cow liveweight (CH_4 / LWt^{-1}), carbon dioxide emissions per day (CO_2) and carbon dioxide emissions per kg of cow liveweight (CO_2 / LWt^{-1}) are presented. Study sub-group 2 commenced and ended the study one week after study sub-group 1.

		CH_4 (g d^{-1})				CH_4 / LWt^{-1} (g kg^{-1})			CO_2 (g d^{-1})				CO_2 / LWt^{-1} (g kg^{-1})		
Cow	Sub-group	Gradient	Intercept	t	P	Gradient	t	P	Gradient	Intercept	t	P	Gradient	t	P
1	2	5.27	278.14	4.49	<0.001	0.008	4.10	<0.001	366.55	10740.24	5.05	<0.001	0.541	4.70	<0.001
2	2	2.40	122.40	6.21	<0.001	0.004	6.05	<0.001	123.80	4610.86	8.92	<0.001	0.231	8.42	<0.001
3	1	5.03	273.53	2.74	<0.001	0.008	2.52	0.03	276.98	10767.88	4.88	<0.001	0.428	4.58	<0.001
4	1	2.14	299.56	0.96	0.35	0.003	0.90	0.38	102.09	13051.08	7.13	0.26	0.165	1.10	0.29
5	2	3.73	284.95	2.81	0.01	0.006	2.72	0.01	230.97	10145.53	5.53	<0.001	0.366	5.28	<0.001
6	2	2.11	402.69	1.70	0.23	0.003	1.11	0.28	97.98	20971.03	0.22	0.83	0.143	0.19	0.85
7	2	5.33	155.03	5.46	<0.001	0.008	5.12	<0.001	194.52	6097.98	4.79	<0.001	0.302	4.39	<0.001
8	1	1.33	115.84	2.64	0.02	0.003	2.64	0.02	89.41	5328.81	4.99	<0.001	0.198	4.98	<0.001
9	2	1.01	509.46	0.47	0.65	0.000	0.11	0.92	134.50	20052.80	1.21	0.24	0.184	0.90	0.38
10	1	0.25	332.71	0.31	0.76	0.000	-0.17	0.87	8.76	13434.07	0.22	0.83	-0.012	-0.16	0.88
11	1	2.58	267.75	2.26	0.04	0.004	2.19	0.04	144.69	10100.34	2.62	<0.001	0.247	5.51	<0.001
1	1	4.9	221	6.	<0.	0.0	5.	<0.	189	118	5.	<0.	0.2	4.	<0.

2	4	.55	2 1	00 1	08	2 3	00 1	.60	01.5 8	2 1	00 1	84	2 9	00 1
A ll	3.0 1	272 .21	7. 4 3	<0. 00 1	0.0 05	6. 6 1	<0. 00 1	164 .13	114 29.4 1	4. 0 0	<0. 00 1	0.2 58	3. 6 4	<0. 00 1

Table 5. Mean rank and the standard deviation of rank (SD) for each individual cow according to their methane (CH_4) and carbon dioxide (CO_2) emissions over the study period. The highest emitting cow is rank 1 and lowest emitting cow is rank 12.

	CH_4 (g d^{-1})		CO_2 (g d^{-1})	
Cow	Mean rank	SD	Mean rank	SD
1	7.0	2.1	7.4	2.2
2	1.5	0.6	1.7	2.0
3	6.4	2.5	7.0	2.3
4	5.5	2.5	6.1	2.6
5	6.3	1.7	5.7	1.7
6	8.6	2.1	8.1	2.4
7	2.6	3.0	2.2	2.2
8	3.0	1.8	2.8	1.5
9	9.8	1.2	9.4	1.3
10	6.5	2.1	6.3	2.4
11	5.5	2.0	4.4	1.6
12	5.0	2.0	6.9	2.0

Public interest statement

Agriculture is a major contributor to the greenhouse gas emissions that have been linked with climate change. Ruminant livestock, such as dairy cows, produce the potent greenhouse gas, methane, which predominantly comes from their breath. One way of reducing the amount of methane produced by dairy cows is to change their diets. We tested how much methane production changed when two groups of dairy cows were moved onto a diet of grazed grass from a diet of barley straw. We measured that methane production increased by an average of 42%, six weeks after the dietary change. However, methane production may not have reached maximum values during our experiment. Some individual cows produced four times more methane than others. Our results indicated that methane production may be reduced if low emitting cows are selected. We conclude that greenhouse gas models must include the time taken to adjust to new feeding regimes.

About the authors

The lead author (Dr Mark Lee) is an Early Career Research Fellow in Natural Capital and Plant Health at the Royal Botanic Gardens Kew. He is currently leading innovative research projects using novel approaches to investigate the sustainable intensification of soft fruit, cereal crop and livestock production systems. In particular, he is interested in the interactions between forage crops, livestock productivity and greenhouse gas emissions. The Royal Botanic Gardens Kew is an internationally renowned centre for plant sciences, producing research on some of the biggest issues facing the global population. The experimental work for this research article was conducted at Scotland’s Rural College (SRUC). SRUC delivers comprehensive skills, education and business support for Scotland’s land-based industries, founded on world class and sector-leading research, education and consultancy.